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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

DATE: August 22, 2007

TXR#: 0054658

SUBJECT: **Clodinafop-propargyle: Developmental Neurotoxicity Study- Rat**
PC Code: 125203, DP Barcode: D338839

FROM: Yung G. Yang, Ph.D.
Toxicology Branch
Health Effects Division (7509P)

THRU: Jess Rowland, Chair
DNT Workgroup
Health Effect Division (7509P)

TO: Paula Deschamp, Chief
Registration Action Branch 3
Health Effect Division (7509P)

and

James Stone/Joanne Miller
Herbicide Branch
Registration Division (7505P)

CONCLUSION: The HED has reviewed all doses including the submitted additional brain morphometry data from the mid- and low-dose groups for the developmental neurotoxicity study for clodinafop-propargyl and determined that most of the brain morphometric changes were not to be treatment related due to lack of dose , time, and/or sex-response and the mean values were within the range of the historical control means. However, the increase seen in the level 4 corpus callosum thickness in PND 63 females at the high dose was considered to be treatment-related. **The revised offspring LOAEL is 500 ppm (44.0 mg/kg/day) based on decreased pup body weights and body weight gains, increased liver weight, decreased auditory response on PND 23 and increased thickness of corpus callosum in females on PND 63. The offspring NOAEL is 100 ppm (9.0 mg/kg/day).**

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BACKGROUND

The developmental neurotoxicity study (MRID 46012925, 46012948) has been previous reviewed by HED (TX No. 0053649 & 0052183). During their evaluation of this study, HED's DNT Work Group requested brain morphometrics data from the low- and the mid-dose groups since marked increases in the thickness of corpus callosum were seen at the high dose females at post natal day 63 (TXR# 0054358). In response, the registrant has conducted and submitted additional brain morphometry data to fulfill this requirement (MRID 47085301).

RESPONSE

The HED has reviewed the submitted additional brain morphometry data from the mid- and low-dose groups for the developmental neurotoxicity study for clodinafop-propargyl. A revised Data Evaluation Record (DER) that includes the evaluation and conclusions on the additional brain morphometry data submitted by the Registrant is attached to this memorandum.

Evaluation of the brain morphometric data at all dose levels showed that several measurements were significantly different ($p \leq 0.05$) from the concurrent controls. However, most of the changes were determined not to be treatment related due to lack of dose-, time, and/or sex-response and the mean values were within the range of the historical control means. In contrast, the 24% increase ($p < 0.01$) seen in the level 4 corpus callosum thickness in PND 63 females at the high dose was considered to be treatment-related due to the magnitude of the increase and changes in linear morphometric measurements can reflect alterations in the development of particular brain regions that are associated with functional deficits.

With consideration of additional brain morphometry data, the offspring LOAEL is revised to 500 ppm (44.0 mg/kg/day) based on decreased pup body weights and body weight gains, increased liver weight, decreased auditory response on PND 23 and increased thickness of corpus callosum in females on PND 63. The offspring NOAEL is 100 ppm (9.0 mg/kg/day).

An executive summary is as follows.

EXECUTIVE SUMMARY: The developmental neurotoxicity study has been previous reviewed by HED (TX No. 0053649 & 0052183). During their evaluation of this study, HED's DNT Work Group requested brain morphometrics data from the low- and the mid-dose groups since marked increases in the thickness of corpus callosum were seen at the high dose females at post natal day 63 (TXR# 0054358). In response, the registrant has conducted and submitted additional brain morphometry data to fulfill this requirement (MRID 47085301). This revised Data Evaluation Record (DER) includes the evaluation and conclusions reached on the additional brain morphometry data submitted by the Registrant.

In a developmental neurotoxicity study (MRID 46012925, 46012948, 47085301), Clodinafop-propargyl (94.2% a.i) was administered in the diet to pregnant Alpk:APfSD Wistar-derived rats (30/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 20, 100, or 500 ppm (equivalent to 0/0, 1.8/3.5, 9.0/18.0, and 44.0/85.5 mg/kg/day [gestation/lactation]). Dams were allowed to deliver naturally and were killed on LD 29. On postnatal day (PND) 5, litters were standardized to 8 pups/litter; the remaining offspring and dams were sacrificed and discarded without further examination. Subsequently, 1 pup/litter/group (10 pups/sex/dose) were allocated to subsets for FOB, motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination. Positive control data were submitted and considered acceptable.

For maternal toxicity, no treatment-related effects were observed on mortality, clinical signs, body weight, body weight gain, food consumption, FOB, reproductive performance and postmortem examinations. **The maternal NOAEL is 500 ppm (44.0 mg/kg/day). The maternal LOAEL was not established.**

For offspring, no significant treatment-related differences in live litter size, post-natal survival or sex ratios were observed. Pre-weaning pup body weights were decreased (↓ 9-19%; $p \leq 0.01$) in both sexes at 500 ppm beginning on PND 12 through PND 22. Minor decreases (↓ 2-5%; $p \leq 0.05$) in body weights were noted in the 100 ppm males on PND 22 and in the 20 ppm and 100 ppm females on PND 18 and 22. Post-weaning body weights remained decreased (↓ 4-15%; $p \leq 0.01$) in the males through termination on PND 63 and in the females through PND 57. Minor decreases (↓ 2-4%; $p \leq 0.05$) in body weights were noted in the 100 ppm males through PND 50 and in the 100 ppm females through PND 36.

For sexual maturation, increased ($p \leq 0.05$) time to preputial separation was noted in the 100 and 500 ppm male pups when compared to controls (45.5-45.7 days treated vs 44.7 days controls). Time to vaginal opening was delayed in the 500 ppm females (35.6 days) compared to controls (34.6 days). However, it was not considered biologically significant since these results were within the historical control ranges.

Motor activity assessment was determined to be inadequate because of the lack of habituation in the female control groups confounded the interpretation of the effects seen in the low and mid dose group female offspring.

Treatment-related effects on auditory startle reflex were observed at high dose on PND 23 in peak amplitude with supportive responses observed at the mid-dose. There were no treatment-related differences in the water maze tests. Swimming time in the Y-maze was sporadically increased or decreased during several trials in the 20 and 100 ppm males and in all dose groups in the females. However, these differences were transient and unrelated to dose. Swimming time in the straight channel was increased (↑ 23%; $p \leq 0.05$) in the 500 ppm females compared to controls during the memory phase (PND 62). There were no treatment-related differences in the percent of successful swimming trials in either sex at either time point.

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For postmortem examinations, absolute liver weights were increased (↑20-36%; p≤0.01) at 500 ppm in males and females at PND 5 and 12. Relative (to body) liver weights were also increased (↑ 26-51%; statistics not performed) in these animals at PND 5 and 12.

Evaluation of the brain morphometric data at all dose levels showed several measurements that were significantly different (p≤0.05) from the concurrent controls. However, most of the changes were determined not to be treatment related due to lack of dose-, time, and/or sex-response and the mean values were within the range of the historical control means. In contrast, the 24% increase (p <0.01) seen in the level 4 corpus callosum thickness in PND 63 females at the high dose was considered to be treatment-related due to the magnitude of the increase and changes in linear morphometric measurements can reflect alterations in the development of particular brain regions that are associated with functional deficits. The corpus callosum captures several major developmental processes subject to environmental insult, including myelination, axonal growth and pruning. For example, a loss of the pruning process may result in a larger corpus callosum than is typical (Altman 1987; Rodier 1988, 1995, 2004; Rodier et al 1997).

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This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 due to the inadequacies in the assessment of motor activity in the offspring and the pending review of the positive control data.

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EPA Reviewer: Yung G. Yang, Ph.D.
Toxicology Branch, Health Effects Division (7509P)
EPA Secondary Reviewer: Jess Rowland, Chief
Science Information Management Branch,
Health Effects Division (7509P)

Signature: Yung G. Yang
Date: 8/22/2007
Signature: Jess Rowland
Date: 8/22/07

Template version 11/01

TXR#: 0054658

DATA EVALUATION RECORD
Revised TXR# 0053649 & 0052183 with supplemental data

STUDY TYPE: Developmental Neurotoxicity Study – Rat; OPPTS 870.6300, OECD 426

PC CODE: 125203 **DP BARCODE:** D338839

TEST MATERIAL (PURITY): Clodinafop-propargyl technical (94.2% a.i.)

SYNONYMS: (R)-2-(4-((5-chloro-3-fluoro-2-pyridinyl)oxy)phenoxy)propionic acid 2-propynyl ester

CITATION: Milburn, G.M. (2007) Clodinafop-Propargyl: Supplement to developmental neurotoxicity study in rats (MRID 46012925). Central Toxicology Laboratory, Cheshire, UK. Laboratory Report # RR0938-REG-S1, Study #: WR0655 Supplement to RR0938, March 14, 2007. MRID 47085301. Unpublished.

Milburn, G.M. (2003) Clodinafop-Propargyl: developmental neurotoxicity study in rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Study Id.: RR0938, May 2, 2003. MRID 46012925. Unpublished

Milburn, G.M. (2003) Clodinafop-Propargyl: preliminary developmental neurotoxicity study in rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Study Id.: RR0937, January 17, 2003. MRID 46012948. Unpublish

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Greensboro, NC

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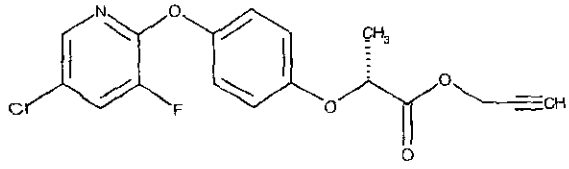
COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Clodinafop-propargyl

Description:	Beige/brown solid
Lot/Batch #:	P903007
Purity:	94.2% a.i.
Compound Stability:	The test substance was stable in the diet for up to 21 days at room temperature.
CAS # of TGAI:	105512-06-9
Structure:	

2. Vehicle and/or positive control: Diet

3. Test animals (P)

Species:	Rat	
Strain:	Alpk:AP$_f$SD (Wistar-derived)	
Age at GD 1:	10-12 weeks	
Wt. at GD 1:	216-285 g (females)	
Source:	Rodent Breeding Unit (RBU), Alderly Park, Macclesfield, Cheshire, UK	
Housing:	P dams were housed individually until parturition and then with their litters until PND 29 in solid plastic cages with wood shaving bedding. F1 animals were housed 4/cage (separated by sex) in wire mesh cages.	
Diet:	CT1 diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum*, except during behavioral testing	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70%
	Air changes:	≥15/hour
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	6 days prior to the start of dosing (i.e., received by the breeding laboratory on GD 1)	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 07/30/02 End: 02/07/03

2. Study schedule: The test substance was administered to the maternal animals from gestation day (GD) 7 through lactation day (LD) 22. F1 pups were selected on post-natal day (PND) 5, weaned on PND 29, and assigned to subgroups in order to evaluate behavioral abnormalities, motor activity, auditory startle response, learning and memory, and neuropathology, including morphometry and brain weights. Liver weights were also determined for control and 500 ppm groups on PND 5 and 12, as an indication of exposure to the test substance during early and mid lactation.

3. Mating procedure: The maternal animals were mated by the supplier and examined for the presence of spermatozoa in a vaginal smear to verify positive mating. Dams were shipped to the performing laboratory the day on which positive mating was found, designated GD 1. Twenty time-mated females were supplied on each of 6 days.

4. Animal assignment: Mated females were randomly assigned, blocked by arrival day, to dose groups as indicated in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 5.

TABLE 1. Study design [a]

Parameter	Dose (ppm)			
	0	20	100	500
Maternal Animals				
No. of maternal animals	30	30	30	30
FOB (GD 10 & 17; LD 2 & 9)	30	30	30	30
Offspring				
FOB (PND 5, 12, 22, 36, 46, & 61)	1pup/litter	1pup/litter	1pup/litter	1pup/litter
Motor activity (PND 14, 18, 22, & 60)	1pup/litter	1pup/litter	1pup/litter	1pup/litter
Auditory startle test (PND 23 & 61)	1pup/litter	1pup/litter	1pup/litter	1pup/litter
Learning and memory (water maze) (PND 21 & 24) (PND 59 & 62)	1pup/sex/litter 1pup/sex/litter	1pup/sex/litter 1pup/sex/litter	1pup/sex/litter 1pup/sex/litter	1pup/sex/litter 1pup/sex/litter
Brain weight and neuropathology [b] (PND 12 & 63)	1pup/litter (10 pups/sex)	1pup/sex/litter (10 pups/sex)	1pup/sex/litter (10 pups/sex)	1pup/sex/litter (10 pups/sex)
Brain cholinesterase determination	NA	NA	NA	NA

a Data obtained from pages 19-25 of MRID 46012925.
b At each sacrifice time, 1 pup/litter was taken to give at least 10 pups/sex/dose.
NA Not applicable

5. Dose selection rationale: A study protocol had been submitted to the Agency and was reviewed and commented by the DNT Workgroup (TXR#014548, April 24, 2001). A preliminary developmental neurotoxicity study in the rat (MRID 46012948) was conducted and submitted concurrently. A summary of this range-finding study is included as Appendix I in this DER.

6. Dosage administration: All doses were administered to maternal animals continuously in the diet from GD 7 through LD 22.

7. Dosage preparation and analysis: Test diets were prepared in 20 kg batches from 500 g premixes prepared for each dose level by mixing the appropriate amount of the test substance with milled diet. Frequency of preparations was not stated, although the test diets were stored for up to 21 days at room temperature. Concentration analyses were performed on diets from all dose groups in the first batch prepared for the study and on two occasions during the study. Additionally in the first batch, homogeneity (top, middle, and bottom) and stability (21 days at room temperature) of the test substance in the diet were verified.

Results - **Homogeneity:** 95.0-108.4% nominal; 1.3-4.0% CV

Stability: 88.1-91.7% initial concentration after 21 days at room temperature.

Concentration: 95.0-108.4% nominal

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: Cage-side observations were conducted twice daily. Detailed clinical examinations were performed at the same time that body weights were measured.

All rats were examined outside the home cage on GD 10 and 17 and LD 2 and 9 using a functional observation battery of tests which included, but were not limited to, the following:

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalmus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Body weights were recorded on: GD 1, 7, 15, and 22; LD 1, 5, 8, 12, 15, and 22; and at termination. Food consumption (g/rat/day) was reported for each week during gestation and for LD 1-5, 5-8, 8-12, 12-15, 15-18, 18-21, and 21-23.

b. Offspring

1) Litter observations: Each litter was examined as soon as possible (always within 24 hours) after completion of parturition (PND 1). The sex of each pup was recorded on PND 1 and 5. The body weight and clinical condition of each pup was recorded on PND 1, 5, 12, 18, and 22. In addition, daily checks for dead or abnormal pups were conducted.

On PND 5, litters were standardized to 8 pups (4/sex/litter, as near as possible), and selection of the F1 generation was carried out using litters comprised of 7 or 8 pups with at least 3 males and 3 females. Pups not selected for the F1 generation on PND 5 were killed and discarded.

2) Developmental landmarks: Beginning on PND 29, the selected F1 females were examined daily to determine the age at which vaginal opening occurred. Beginning on PND 41, the selected F1 males were examined daily to determine the age at which balanopreputial separation occurred. Body weights on the day of balanopreputial separation/vaginal patency were not reported.

3) Postweaning observations: Cage-side observations for mortality and clinical signs of toxicity were performed daily. Body weight measurements and detailed physical examination of each rat was conducted on PND 29, 36, 43, 50, 57, and prior to termination on PND 63.

4) Neurobehavioral evaluations

i) Functional observational battery (FOB): At least 10 rats/sex/dose (one male or one female per litter) were examined on PND 5, 12, 22, 36, 46, and 61 using a functional observational battery of tests. The FOB was conducted by a technician who was "blind" to the treatment groups and was comprised of the same parameters examined in the maternal rats.

ii) Motor activity testing: Motor activity was evaluated in at least 10 rats/sex/dose (one male or one female per litter) on PND 14, 18, 22, and 60, using an automated recording apparatus (details not provided) which recorded small and large movements as an activity count. Each 50 minute session was divided into 10 subsessions of five-minute duration. The same offspring were evaluated at each time point, and each animal was returned to the same activity monitor when trials were repeated.

iii) Auditory startle reflex habituation: An auditory startle habituation test was conducted on at least 10 rats/sex/dose (one male or one female per litter) on PND 23 and 61, using an automated recording apparatus (details not provided). Fifty trials (repetitions) were performed on each animal per each day of testing. The mean response amplitude and time to maximum amplitude were calculated on each block of 10 trials (5 blocks of 10 trials per day of testing).

iv) Learning and memory testing: Associative learning and memory were tested in one rat/sex/litter/dose on PND 21 and 24 and another rat/sex/litter/dose on PND 59 and 62. The test used a Y-shaped water maze with one escape ladder. The time taken to find the escape ladder was recorded for each trial. Animals were given 6 trials on either PND 21 or 59 (learning phase) and were retested three days later (PND 24 or 62) using the same procedures (memory phase). In order to assess swimming speed, each animal completed one trial in a straight channel immediately following the six trials during the learning phase and the memory phase. Swimming times for the Y-maze and straight channel were reported. The percentage of successful trials in the Y-maze was calculated for each animal. The criterion for a successful trial was a time less than a given cut-off value. Cut-off values of 3, 4, 5, 6, 7, 8, 9, and 10 seconds and 1x, 1.5x, and 2.5x the straight channel time were used.

5) Cholinesterase determination: Cholinesterase activity was not determined.

6) Pharmacokinetic data: Pharmacokinetics were not evaluated in this study.

2. Postmortem observations

a. Maternal animals: Two females which failed to litter (one control and one 20 ppm) were sacrificed by over exposure to halothane Ph. Eur. vapor followed by exsanguination and were subjected to a macroscopic examination (including an examination of the uterus to confirm pregnancy status). Females with total litter loss or with litters not required for selection were sacrificed and discarded. On PND 29, all surviving maternal animals were sacrificed and discarded without examination.

b. Offspring: Prior to selection on PND 5, any pups found dead or killed intercurrently were given a macroscopic visceral examination and discarded. Any pups that died following PND 5 were not examined because they were partially or completely cannibalized. On PND 5, one pup/sex/litter from animals in the control and 500 ppm groups not selected to continue the study was sacrificed by cervical dislocation followed by decapitation, and the liver was excised and weighed in order to demonstrate exposure to the test substance.

On PND 12, at least 10 pups/sex/group (one male or one female per litter) were killed by a rising concentration of carbon dioxide. The brain was immediately fixed in 10% neutral buffered formol saline and weighed after at least 24 hours fixation. Additionally for animals in the control and 500 ppm groups, the liver was weighed.

On PND 63, at least 10 pups/sex/group (one male or one female per litter) were killed as described for PND 12, except that after weighing, the brain was fixed and stored. A further 10 rats/sex/group were deeply anesthetized by intraperitoneal injection of sodium pentobarbitone and killed by perfusion fixation with formol saline. The brain was weighed.

The HED DNT Workgroup reviewed the study and determined that the brain morphometric changes observed in the PND 63 females at the high dose are treatment-related. Therefore, the

level 4 corpus callosum thickness data from the mid- and low-dose groups are required for both PND 12 and 63. In addition, other brain morphometric measurements in which statistical significant differences were seen should be evaluated in male and female mid- and low-dose groups (TXR# 0054358, September 20, 2006). In response, the Registrant submitted supplemental study on brain pathology and morphometry measurements for group 2 and 3 offspring killed on PND 12 or 63 under the same protocol as in the original study (WR0655). In the original study, all brain (groups 2 and 3) had been processed to block during the initial conduct of the study.

The following CHECKED (X) tissues were evaluated for F1 adults:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic Nerve (proximal)
X	Frontal lobe		
X	Parietal lobe		**OTHER**
X	Midbrain with occipital and temporal lobe		Sural Nerve
X	Pons	X	Tibial Nerve (proximal and distal)
X	Medulla oblongata		Peroneal Nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
	SPINAL CORD	X	Lumbar dorsal root fibers
X	Cervical swelling	X	Lumbar ventral root fibers
X	Lumbar swelling	X	Cervical dorsal root ganglion
	OTHER	X	Cervical dorsal root fibers
	Gasserian ganglia with nerve	X	Cervical ventral root fibers
	Pituitary gland		
X	Eyes (with retina and optic nerve)		
X	Skeletal muscle (gastrocnemius)		

The tibial and sciatic nerves were embedded in resin, sectioned, and stained with toluidine blue. The remaining tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All tissues in the control and 500 ppm groups were examined using light microscopy.

D. DATA ANALYSIS

1. Statistical analyses: Data were analyzed using the following statistical procedures:

Parameter	Statistical test
Maternal body weights	Body weights on LD 1 were analyzed by analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used for body weights during gestation using GD 7 body weight as the covariate and for body weights during lactation (other than LD1) using LD 1 as the covariate. ANOVA (or ANCOVA) were followed by Student's t-test, if necessary, for pair-wise comparison of treated groups with controls.

Pup body weight	Body weights on PND 1 and those selected on PND 5 were analyzed by ANOVA. Body weights on subsequent pre-cull days and post-cull days were adjusted for body weights on PND 1 or PND 5, respectively, and analyzed using ANCOVA. ANOVA (or ANCOVA) were followed by Student's t-test, if necessary, for pair-wise comparison of treated groups with controls.
Maternal food consumption, litter size, motor activity, startle response, time to preputial separation, time to vaginal opening, swimming times	ANOVA followed by Student's t-test, if necessary.
Proportions of whole litter loss, pups born live, pups surviving, litters with all pups born live, litters with all pups surviving, male pups, males with preputial separation, females with vaginal opening,	Fisher's Exact Test
Percentages of live born pups, pre-cull pup survival, sex ratio, and successful swimming trials	ANOVA following the double arcsine transformation of Freeman and Tukey, followed by Student's t-test, if necessary.
Brain and liver weights and brain morphology	ANOVA and ANCOVA using the terminal body weight as the covariate followed by Student's t-test, if necessary.

Analyses of lactation (post-natal) body weights, food consumption, litter size, and pup survival are presented excluding whole litter losses. Analyses of live born index are presented including and excluding whole litter losses. All statistical tests were two-sided.

2. **Indices** - Although the formula was not provided, live birth index was reported as the percent of live born pups, both including and excluding whole litter losses. No other indices were reported. The reviewers calculated the following indices and included the data in the summary tables.

Viability index (%) = # pups surviving to PND 5 (pre-cull)/# pups born live x 100

Gestation index (%) = # dams with live pups on the day of birth/# pregnant x 100

3. **Positive control data** - Positive control data on brain morphometry in pups (MRID 46336204) and on FOB, motor activity and morphometry in adult rats, both studies using trimethyltin chloride, (MRID 46336203) were submitted. These studies are under review.

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality and clinical and functional observations:** No treatment-related deaths occurred. Several dams were sacrificed prior to termination because they did not litter, suffered complete

litter loss, or because there were insufficient numbers of pups (required at least 7 pups with at least 3 pups/sex/litter). However, these findings were unrelated to treatment.

There were no treatment-related clinical or functional observations.

2. Body weight and food consumption: There were no treatment-related effects on body weights, body weight gains, or food consumption during gestation (Table 2). Only a minor and transient decrease (↓1%; p≤0.05) in body weights was observed in the 500 ppm dams on GD 15. During lactation, body weights and food consumption were decreased (↓3-9%; p≤0.05) in the 500 ppm dams. Actual body weight gain for the overall lactation period (LD 1-29) did not show significant difference among treated and control groups. It is to be noted that during the lactation period, the food consumption was double compared to the gestation period which meant that the test substance intake (mg/kg) was increased at this period.

TABLE 2. Mean (±SD) maternal body weight (g), body weight gains (g), and food consumption (g/animal/day) [a]

Observations/study interval		Dose (ppm)			
		Control	20	100	500
Gestation					
Body weight	GD 1	253.7 ± 18.0	253.6 ± 19.8	250.4 ± 16.6	254.9 ± 15.1
	GD 7	293.2 ± 18.1	289.6 ± 20.1	288.1 ± 16.6	291.6 ± 16.6
	GD 15 [b]	334.3	335.2	335.9	330.9* (↓1)
	GD 22 [b]	409.2	410.6	413.2	409.1
Body weight gain	(GD 1-22)[c]	158.9	156.0	159.9	155.3
Food consumption	Week 1	22.0 ± 2.6	21.6 ± 3.4	22.2 ± 3.4	21.4 ± 2.5
	Week 2	29.2 ± 3.0	28.9 ± 2.9	29.0 ± 3.2	28.8 ± 3.4
	Week 3	31.9 ± 3.9	31.9 ± 4.0	32.4 ± 4.3	31.2 ± 4.0
Lactation					
Body weight	LD 1	313.0 ± 28.0	312.3 ± 31.0	307.1 ± 22.7	316.3 ± 18.6
	LD 8 [d]	341.2	341.2	337.5	327.8** (↓4)
	LD 12 [d]	355.0	353.6	349.9	338.1** (↓5)
	LD 15 [d]	364.2	363.1	360.4	349.9** (↓4)
	LD 29 [d]	357.7	351.0	350.5	347.7* (↓3)
Body weight gain (LD 1-29)[c]		44.8	38.4	40.3	34.2
Food consumption	LD 8-12	59.3 ± 6.6	58.5 ± 8.0	59.7 ± 8.5	56.2 ± 6.3* (↓5)
	LD 12-15	64.6 ± 5.3	62.6 ± 5.1	62.1 ± 5.4	59.0 ± 6.9** (↓9)

Observations/study interval	Dose (ppm)			
	Control	20	100	500
LD 18-21	75.3 ± 4.9	74.7 ± 6.1	74.7 ± 4.4	71.0 ± 5.5* (↓6)
LD 21-23	86.6 ± 4.9	87.5 ± 8.9	86.5 ± 7.5	81.3 ± 6.7* (↓6)

a Data obtained from Tables 6 through 9 on pages 71-74 in MRID 46012925; n = 20-30. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Means are adjusted based on the body weight on GD 7.
c Calculated by the reviewers from the differences in unadjusted group mean body weights.
d Means are adjusted based on the body weight on LD 1.
* Statistically different from the controls at p≤0.05.
** Statistically different from the controls at p≤0.01.

3. Test Substance Intake: Based on maternal food consumption, body weight, and the nominal concentration in the diet, the mean test substance intake (mg/kg bw/day) during the gestation and lactation periods are presented in Table 3.

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day)[a]

Parameter	Dose (ppm)		
	20	100	500
Gestation	1.8	9.0	44.0
Lactation	3.5	18.0	85.5

a Data were obtained from Appendix H on pages 208-209 in MRID 46012925.

4. Reproductive performance: Reproductive performance was unaffected by the test substance (Table 4).

TABLE 4. Reproductive performance [a]

Observation	Dose (ppm)			
	0	20	100	500
Number mated	30	30	30	30
Number pregnant	29	29	30	30
Number not pregnant	1	1	0	0
Number of litters (# with live born)	26	28	30	29
Gestation index (%) [b]	89.7	96.6	100.0	96.7
Number with complete litter loss	3	1	0	1
Insufficient number or sex ratio of pups [c]	5	3	3	5
Mean (±SD) gestation duration (days)	22.0 ± 0.0	22.0 ± 0.0	22.0 ± 0.0	22.0 ± 0.0
Incidence of dystocia [d]	0	0	0	0

a Data obtained from pages 27, 75, and 77-79 in MRID 46012925.
b Gestation index was calculated by the reviewers from data presented in this table as:

with live born/ # pregnant x 100.

c Sufficient number of pups was defined as at least 3 males and 3 females in a litter of at least 7 pups. Dams and litters not meeting this criteria were sacrificed.

d The reviewers determined that the incidence of dystocia was zero, because both dams that failed to litter were not pregnant (page 162 in MRID 46012925).

5. Maternal postmortem results: No treatment-related findings were noted at necropsy.

B. OFFSPRING

1. Viability and clinical signs: Litter size and viability (survival) results from pups prior to selection on PND 5 are summarized in Table 5. Litter size and survival were not presented after culling on PND 5. However, it was stated that a small number of F1 pups failed to survive to scheduled termination. No treatment-related findings were noted. When whole litter losses are excluded, live birth index was slightly decreased ($p \leq 0.01$) at 500 ppm (97.4%) compared to controls (100%). However, when whole litter losses are included, this effect is not evident and is thus considered unrelated to treatment. There were no treatment-related clinical signs.

TABLE 5. Litter size and viability [a]

Observation	Dose (ppm)			
	Control	20	100	500
Total number born	369	357	378	381
Number born live	356	352	377	371
Number born dead [b]	13	5	1	10
Sex Ratio Day 1 (%)	46.1 ± 17.5	52.3 ± 15.0	51.5 ± 16.3	48.1 ± 16.4
Deaths Days 1-5 [c]	43	20	12	45
Deaths Days 5-22	NR	NR	NR	NR
No. of Litters	29	29	30	30
Mean litter size:				
Day 1	12.5 ± 2.6	12.1 ± 2.8	12.6 ± 2.7	12.3 ± 3.1
Day 5 [d]	12.0 ± 2.5	11.9 ± 2.6	12.2 ± 2.6	11.2 ± 3.2
Day 5 [e]	NR	NR	NR	NR
Day 21	NR	NR	NR	NR
Live birth index (including whole litter loss)	97.1 ± 11.8	98.4 ± 4.9	99.4 ± 3.0**	97.5 ± 4.8
Live birth index (excluding whole litter loss)	100.0 ± 0.0	99.1 ± 3.5	99.4 ± 3.0	97.4 ± 4.9**
Viability index (%) [f]	87.9	94.3	96.8	87.9
Lactation index (%)	NR	NR	NR	NR

a Data obtained from Tables 11 through 15 on pages 76-81 in MRID 46012925.

b Calculated by the reviewers from data presented in this table.

c Calculated by the reviewers as # born live (found in this table) - # surviving PND 1-5 (found in Table 14 on page 80 of MRID 46012925).

d Before standardization (culling).

e After standardization (culling).

f Calculated by the reviewers as # surviving PND 1-5 (found in Table 14 on page 80 of MRID 46012925) divided by # born live (found in this table) x 100.

NR Not reported

2. Body weight: Pre-weaning pup body weights were decreased (↓9-19%; p≤0.01) in both sexes at 500 ppm beginning on PND 12 (Table 6a). Minor decreases (↓2-5%; p≤0.05) in body weights were noted in the 100 ppm males on PND 22 and in the 20 ppm and 100 ppm females on PND 18 and 22.

Post-weaning body weights remained decreased (↓4-15%; p≤0.01) at 500 ppm in males through termination on PND 63 and in females through PND 57 (Table 6b). Minor decreases (↓2-4%; p≤0.05) in body weights were noted in the 100 ppm males through PND 50 and in the 100 ppm females through PND 36. No other treatment-related differences in body weights were noted during pre- or post-weaning.

TABLE 6a. Mean (±SD) pre-weaning pup body weights (g) [a]

Post-natal Day	Dose (ppm)							
	0	20	100	500	0	20	100	500
	Males				Females			
1	6.1 ± 0.6	6.0 ± 0.5	6.1 ± 0.5	5.9 ± 0.6	5.7 ± 0.5	5.7 ± 0.5	5.7 ± 0.5	5.6 ± 0.6
5 [b, d]	9.6	9.8	9.7	9.7	9.2	9.3	9.3	9.2
5 [c]	9.5 ± 1.3	9.7 ± 1.1	9.7 ± 0.9	9.3 ± 1.2	9.3 ± 1.3	9.2 ± 1.0	9.1 ± 0.9	9.0 ± 1.2
12 [e]	24.5	24.1	24.3	22.3**(↓9)	23.9	23.6	23.4	21.7**(↓9)
18 [e]	40.0	39.4	39.0	34.3**(↓14)	39.3	38.0*(↓3)	37.3**(↓5)	33.3**(↓15)
22 [e]	55.1	54.3	53.4*(↓3)	44.9**(↓19)	53.5	52.2*(↓2)	51.1**(↓4)	43.5**(↓19)

a Data were obtained from Tables 16 and 20 on pages 82, 83, 116, and 118 in MRID 46012925. Percent difference from controls, calculated by the reviewers, is included in parentheses. n = 29-30 (day 1); n= 26-30 (day 5 pre-cull); n= 21-27 (day 5 post cull); n= 21-27 (days 12-22)
b Before standardization (culling)
c After standardization (culling)
d Means are adjusted based on the body weight on PND 1.
e Means are adjusted based on the body weight on PND 5 (post-cull).
* Statistically different from controls at p≤0.05
** Statistically different from controls at p≤0.01

TABLE 6b. Adjusted mean post-weaning pup body weights (g) [a]

Post-natal Day	Dose (ppm)							
	0	20	100	500	0	20	100	500
	Males				Females			
29	96.9	96.1	93.8**(↓3)	82.5**(↓15)	90.6	89.8	87.4**(↓4)	77.3**(↓15)
36	153.2	152.0	148.0**(↓3)	135.6**(↓11)	132.9	133.2	129.1*(↓3)	119.9**(↓10)
43	211.9	211.0	206.7*(↓2)	194.3**(↓8)	169.4	169.9	165.7	157.3**(↓7)
50	267.6	266.2	260.4*(↓3)	245.5**(↓8)	193.2	193.5	189.3	181.8**(↓6)
57	324.7	321.9	317.0	302.1**(↓7)	212.5	213.7	209.0	203.4**(↓4)

63	362.6	357.5	355.4	337.7**(↓7)	221.8	223.4	219.6	215.2

a Data were obtained from Table 20 on pages 116-119 in MRID 46012925. Means are adjusted based on the body weight on PND 5 (post-cull). Percent difference from controls, calculated by the reviewers, is included in parentheses n = 21-27 for both sexes for all PND

* Statistically different from controls at p≤0.05

** Statistically different from controls at p≤0.01

3. Developmental landmarks

a) Sexual maturation: Sexual maturation data are presented in Table 7. Increased (p≤0.05) time to preputial separation was noted in the 100 and 500 ppm male pups when compared to controls (45.5-45.7 days treated vs 44.7 days controls). Time to vaginal opening was delayed (p≤0.01) in the 500 ppm females (35.6 days) compared to controls (34.6 days); The delays in sexual maturation could be attributed to decrease in body weights. Although these decreases showed statistical significance, they were not considered biologically significant since the duration of delays were within the historical control ranges.

TABLE 7. Mean (±SD) age of sexual maturation (days) [a]

Parameter	Dose (ppm)			
	0	20	100	500
N (M/F)	21/21	25/25	27/27	24/24
Preputial separation (males)	44.7 ± 0.8	44.8 ± 0.9	45.5 ± 1.0**	45.7 ± 0.8**
Body Weight (g) at criterion	211.9	211.0	206.7*(↓2)	194.3**(↓8%)
Vaginal opening (females)	34.6 ± 0.7	34.5 ± 0.9	35.0 ± 0.9	35.6 ± 1.3**
Body Weight (g) at criterion	132.9	133.2	129.1*(↓3%)	119.9**(↓0%)

a Data were obtained from Table 21 on pages 120-121 in MRID 46012925.

* Statistically different from controls at p≤0.05

** Statistically different from controls at p≤0.01

b) Physical landmarks: Physical landmarks were not evaluated.

4. Behavioral assessments

a) Functional observational battery: No abnormalities were detected in any of the parameters examined in the functional observational battery at any time point in either sex.

b) Motor activity: There were no treatment-related effects on total activity in males or females during any of the time points (Table 8a). The total mean number of movements more than doubled between PND 14 and 22 in all groups. Motor activity was increased (↑397%; p≤0.05) in the 500 ppm males on PND 14 during the 46-50 minute sub-session (Table 8b). There were no other significant differences in the sub-session motor activity data in the males. During several sub-sessions on PND 18 and 22 in the females, motor activity was decreased (↓41-82%; p≤0.05) in the 20, 100, and/or 500 ppm groups compared to controls (Table 8c). It can not be determined whether the decrease in motor activity in the low and mid-dose group PND 22 females is treatment-related. There was

no dose-response in this decrease because: 1) values were similar between low and mid dose groups and 2) the values were the same between the control and high dose. The decrease occurred in females on PND 22 only; a similar effect was not seen in males. In addition, there appears to be problems with the control habituation data. There was no habituation in PND60 female controls and little habituation in PND 22 female controls; similar problems were not seen in male habituation data. This lack of habituation was also seen at the high dose, but this change can not be compared to controls since no habituation was seen there either.

TABLE 8a. Mean (±SD) motor activity data (total number of movements/50 minutes) [a]

Test Day	Dose (ppm)			
	0	20	100	500
Males				
PND 14	167.0 ± 121.1	148.5 ± 161.3	147.4 ± 136.2	152.4 ± 93.5
PND 18	233.7 ± 153.5	150.9 ± 135.7	250.2 ± 198.1	158.0 ± 112.4
PND 22	370.7 ± 202.4	407.6 ± 194.4	358.6 ± 160.4	355.2 ± 127.3
PND 60	516.3 ± 131.0	538.1 ± 146.0	493.7 ± 135.9	499.5 ± 126.9
Females				
PND 14	144.0 ± 149.9	148.0 ± 124.3	114.6 ± 103.5	171.6 ± 188.6
PND 18	265.4 ± 234.2	206.3 ± 105.3	193.5 ± 142.1	224.0 ± 152.6
PND 22	481.5 ± 163.5	334.3 ± 167.0* (↓31)	347.8 ± 114.6* (↓28)	430.2 ± 151.7
PND 60	597.2 ± 72.2	592.1 ± 127.0	568.9 ± 83.0	591.1 ± 108.3

a Data were obtained from Table 22 on pages 122-129 in MRID 46012925; n = 10-14. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

TABLE 8b. Mean (± SD) sub-session motor activity in males (# movements/5 minute sub-session) [a]

Interval (min)	Dose (ppm)			
	0	20	100	500
Day 14				
1-5	23.6 ± 19.2	20.5 ± 29.5	31.7 ± 28.4	24.0 ± 18.5
6-10	19.3 ± 21.7	26.2 ± 36.4	20.4 ± 21.3	15.4 ± 14.8
11-15	27.4 ± 24.0	15.6 ± 21.3	19.9 ± 26.5	16.4 ± 14.3
16-20	24.0 ± 20.7	7.4 ± 14.6	13.8 ± 19.5	33.0 ± 26.7
21-25	19.9 ± 16.5	14.4 ± 17.0	11.1 ± 15.2	16.1 ± 18.9
26-30	10.2 ± 18.2	25.4 ± 27.2	11.4 ± 18.1	11.7 ± 15.0
31-35	12.9 ± 13.3	11.8 ± 14.6	5.9 ± 10.4	10.2 ± 13.0
36-40	17.2 ± 20.6	9.2 ± 20.2	11.6 ± 18.0	4.7 ± 9.4
41-45	9.3 ± 7.3	11.4 ± 19.4	13.8 ± 21.5	5.1 ± 6.3
46-50	3.2 ± 3.2	6.7 ± 7.1	7.9 ± 16.5	15.9 ± 19.2* (↑397)

Interval (min)	Dose (ppm)			
	0	20	100	500
Day 18				
1-5	25.9 ± 19.1	14.8 ± 10.6	35.6 ± 23.7	18.3 ± 17.1
6-10	27.3 ± 20.8	17.0 ± 17.9	34.3 ± 30.1	19.6 ± 17.9
11-15	18.7 ± 22.2	15.8 ± 19.2	26.9 ± 26.3	7.8 ± 8.5
16-20	22.4 ± 25.3	17.6 ± 19.0	27.0 ± 25.6	15.1 ± 15.8
21-25	14.8 ± 19.0	10.4 ± 16.9	19.4 ± 22.5	19.5 ± 21.1
26-30	19.7 ± 18.1	18.2 ± 24.7	18.2 ± 20.4	11.6 ± 14.7
31-35	34.6 ± 25.2	18.7 ± 24.9	18.1 ± 24.6	18.8 ± 23.7
36-40	24.9 ± 30.2	15.7 ± 23.3	24.5 ± 24.3	18.8 ± 21.3
41-45	23.1 ± 26.0	14.5 ± 24.3	24.1 ± 29.0	11.2 ± 22.4
46-50	22.3 ± 28.2	8.2 ± 18.4	22.1 ± 24.5	17.2 ± 20.8
Day 22				
1-5	55.3 ± 16.6	49.5 ± 16.0	58.4 ± 19.6	51.5 ± 15.5
6-10	39.7 ± 16.3	46.4 ± 24.1	43.3 ± 23.6	42.1 ± 22.5
11-15	36.1 ± 23.9	34.8 ± 34.4	44.1 ± 28.2	38.3 ± 16.4
16-20	31.5 ± 29.4	39.5 ± 28.2	27.0 ± 25.4	42.1 ± 21.7
21-25	32.3 ± 33.6	25.9 ± 25.5	26.8 ± 25.1	30.5 ± 22.5
26-30	31.7 ± 34.5	34.7 ± 27.0	32.3 ± 29.0	38.6 ± 24.2
31-35	40.1 ± 35.5	53.1 ± 33.6	38.9 ± 35.5	37.2 ± 26.4
36-40	38.3 ± 28.6	42.8 ± 35.4	32.0 ± 30.7	25.5 ± 22.9
41-45	39.9 ± 33.2	40.5 ± 30.2	26.6 ± 26.3	27.0 ± 28.7
46-50	25.8 ± 26.0	40.5 ± 33.2	29.1 ± 30.9	22.4 ± 21.6
Day 60				
1-5	65.6 ± 9.7	63.7 ± 11.8	68.6 ± 8.6	63.7 ± 9.9
6-10	67.5 ± 8.5	62.4 ± 12.0	62.9 ± 16.0	58.4 ± 15.2
11-15	59.3 ± 20.3	60.6 ± 17.2	59.5 ± 13.0	59.6 ± 16.5
16-20	54.6 ± 23.0	59.5 ± 11.5	56.5 ± 16.3	56.8 ± 19.3
21-25	60.2 ± 10.9	52.0 ± 18.2	46.4 ± 27.3	46.1 ± 20.4
26-30	43.5 ± 29.9	49.3 ± 22.6	44.9 ± 25.2	49.5 ± 25.3
31-35	44.3 ± 27.5	49.7 ± 23.4	43.0 ± 25.1	45.5 ± 26.5
36-40	47.4 ± 31.3	50.3 ± 26.8	35.3 ± 28.8	39.8 ± 28.6
41-45	37.2 ± 28.1	48.2 ± 29.5	34.9 ± 32.8	36.3 ± 28.6
46-50	36.7 ± 29.5	42.4 ± 25.8	41.7 ± 26.6	43.9 ± 20.7

a Data were obtained from Table 22 *on* pages 122-129 in MRID 46012925; n = 10-14. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at $p \leq 0.05$

TABLE 8c. Mean (± SD) sub-session motor activity in females (# movements/5 minute sub-session) [a]

Interval (min)	Dose (ppm)			
	0	20	100	500
Day 14				
1-5	15.0 ± 20.6	28.3 ± 22.1	22.5 ± 22.0	29.5 ± 23.9
6-10	16.9 ± 23.2	19.5 ± 18.7	13.6 ± 23.1	21.6 ± 22.5
11-15	18.4 ± 20.9	9.8 ± 18.0	13.6 ± 20.6	20.0 ± 29.3
16-20	16.0 ± 20.9	15.0 ± 19.5	13.7 ± 13.7	13.0 ± 22.2
21-25	9.2 ± 11.3	13.4 ± 11.1	11.4 ± 23.2	14.4 ± 19.0
26-30	9.3 ± 13.7	14.0 ± 18.0	13.7 ± 24.0	15.9 ± 26.7
31-35	18.7 ± 24.9	10.8 ± 17.9	6.3 ± 8.6	9.9 ± 17.7
36-40	18.4 ± 24.4	17.6 ± 29.9	7.1 ± 15.9	18.3 ± 28.5
41-45	12.2 ± 18.9	14.8 ± 22.9	8.8 ± 16.0	16.2 ± 27.7
46-50	10.0 ± 19.0	4.8 ± 6.7	3.8 ± 5.1	12.8 ± 20.9
Day 18				
1-5	26.3 ± 22.5	32.6 ± 16.5	33.8 ± 22.0	28.1 ± 21.0
6-10	33.0 ± 36.1	27.6 ± 18.6	31.6 ± 24.7	30.1 ± 24.4
11-15	23.1 ± 24.1	20.2 ± 19.5	23.6 ± 22.7	22.7 ± 20.3
16-20	38.5 ± 33.0	17.7 ± 13.4* (↓54)	12.5 ± 13.7** (↓68)	20.5 ± 18.3* (↓47)
21-25	23.1 ± 26.1	25.9 ± 20.4	15.3 ± 22.0	29.5 ± 24.9
26-30	17.3 ± 25.5	19.6 ± 20.0	14.8 ± 19.7	20.6 ± 24.8
31-35	22.2 ± 29.8	25.4 ± 21.0	12.7 ± 21.9	17.5 ± 20.2
36-40	30.0 ± 36.0	13.1 ± 17.2	5.4 ± 14.9* (↓82)	15.8 ± 17.0
41-45	28.0 ± 34.4	14.1 ± 21.1	17.9 ± 23.1	19.5 ± 21.2
46-50	24.0 ± 33.5	10.3 ± 19.5	25.8 ± 27.6	19.7 ± 23.7
Day 22				
1-5	59.1 ± 22.2	49.7 ± 20.0	53.2 ± 15.4	53.8 ± 19.7
6-10	42.3 ± 26.2	39.6 ± 23.9	37.8 ± 23.4	53.5 ± 20.5
11-15	31.8 ± 25.3	24.3 ± 23.6	34.0 ± 26.1	49.1 ± 20.0
16-20	43.4 ± 31.2	40.8 ± 28.3	33.2 ± 18.7	43.9 ± 25.7
21-25	52.5 ± 18.3	36.8 ± 27.4	32.8 ± 29.1	36.1 ± 29.7
26-30	50.3 ± 21.7	36.7 ± 24.3	34.5 ± 24.6	42.2 ± 26.3
31-35	55.2 ± 20.9	37.6 ± 28.5	42.2 ± 26.6	37.4 ± 28.1
36-40	53.3 ± 22.0	30.8 ± 26.3* (↓42)	31.6 ± 27.2* (↓41)	34.7 ± 25.4
41-45	53.0 ± 24.4	18.8 ± 24.6** (↓65)	28.5 ± 24.9* (↓46)	42.3 ± 31.9
46-50	40.8 ± 30.3	19.3 ± 28.4	20.0 ± 23.0	37.3 ± 29.4
Day 60				
1-5	63.4 ± 11.8	61.7 ± 12.8	60.4 ± 10.9	64.1 ± 10.9
6-10	60.9 ± 13.6	62.6 ± 10.7	63.2 ± 11.7	67.2 ± 11.7

Interval (min)	Dose (ppm)			
	0	20	100	500
11-15	60.6 ± 9.3	68.1 ± 9.2	60.5 ± 12.0	62.9 ± 11.6
16-20	64.4 ± 8.3	57.3 ± 16.4	55.8 ± 13.2	61.6 ± 9.5
21-25	63.3 ± 8.4	56.5 ± 20.1	56.2 ± 12.4	57.9 ± 19.7
26-30	58.6 ± 12.3	55.5 ± 24.7	51.2 ± 16.6	58.0 ± 14.8
31-35	57.1 ± 11.5	55.9 ± 23.4	58.1 ± 13.8	50.5 ± 14.0
36-40	55.3 ± 17.9	57.6 ± 25.5	57.5 ± 15.3	65.1 ± 14.8
41-45	54.5 ± 19.9	59.2 ± 17.0	52.5 ± 17.5	60.7 ± 23.6
46-50	59.2 ± 21.4	57.6 ± 23.4	53.4 ± 20.1	43.1 ± 28.1

a Data were obtained from Table 22 on pages 122-129 in MRID 46012925; n = 10-14. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

** Statistically different from controls at p≤0.01

c) **Auditory startle reflex habituation:** Treatment-related effects on auditory startle reflex were observed at high dose on PND 23 in peak amplitude with supportive evidence observed at the mid-dose (Table 9a). Data, recorded for the five blocks of 10 repetitions/block, indicated that habituation patterns were normal.

TABLE 9a. Mean (± SD) auditory startle reflex peak amplitude (V) [a]

Post-natal Day	Repetition	Dose (ppm)			
		0	20	100	500
		Males			
PND 23	1-10	379.6 ± 98.8	439.3 ± 149.2	372.9 ± 99.2	302.5 ± 122.8
	11-20	266.6 ± 64.6	297.7 ± 66.3	297.4 ± 103.2	200.3 ± 64.1* (↓25)
	21-30	233.3 ± 63.1	261.3 ± 53.3	265.9 ± 91.2	176.7 ± 48.0
	31-40	223.7 ± 50.9	235.4 ± 47.0	251.0 ± 72.6	144.6 ± 54.1** (↓35)
	41-50	208.0 ± 55.4	220.7 ± 45.7	221.6 ± 62.8	148.0 ± 51.1* (↓29)
PND 61	1-10	1351.5 ± 370.6	1692.2 ± 563.1	1394.0 ± 525.3	1305.6 ± 393.7
	11-20	898.6 ± 258.7	1053.2 ± 280.6	952.8 ± 367.3	871.5 ± 228.9
	21-30	861.2 ± 346.1	960.0 ± 336.2	846.6 ± 350.7	878.1 ± 324.9
	31-40	769.7 ± 279.9	963.1 ± 350.3	832.4 ± 332.1	758.3 ± 229.3
	41-50	701.9 ± 307.9	929.6 ± 368.5	859.5 ± 356.0	752.9 ± 270.6
		Females			
PND 23	1-10	374.9 ± 121.3	406.3 ± 177.0	353.2 ± 108.2	297.4 ± 62.7
	11-20	332.9 ± 143.1	306.7 ± 130.3	281.0 ± 122.5	232.8 ± 45.9
	21-30	267.5 ± 67.0	294.3 ± 174.7	252.9 ± 165.0	222.7 ± 55.0
	31-40	234.7 ± 58.3	230.1 ± 128.6	190.0 ± 59.2	202.4 ± 53.7
	41-50	226.3 ± 44.1	249.0 ± 140.1	199.1 ± 72.7	189.9 ± 55.6
PND 61	1-10	1064.0 ± 225.1	974.4 ± 222.8	1161.9 ± 442.2	1237.2 ± 321.2

Post-natal Day	Repetition	Dose (ppm)			
		0	20	100	500
	11-20	874.2 ± 170.2	913.2 ± 277.0	1025.6 ± 441.5	963.5 ± 156.5
	21-30	772.0 ± 234.1	831.4 ± 305.2	908.7 ± 588.7	926.2 ± 203.1
	31-40	677.8 ± 270.4	813.2 ± 442.1	816.2 ± 505.0	832.1 ± 315.4
	41-50	652.6 ± 185.4	633.5 ± 378.1	716.1 ± 380.7	798.6 ± 220.6

a Data were obtained from Tables 23 on pages 130-133 in MRID 46012925; n = 10-14. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

** Statistically different from controls at p≤0.01

TABLE 9b. Mean (± SD) time to peak amplitude (ms) [a]

Post-natal Day	Repetition	Dose (ppm)			
		0	20	100	500
		Males			
PND 23	1-10	27.6 ± 6.1	26.8 ± 4.0	30.9 ± 7.5	28.3 ± 4.8
	11-20	21.8 ± 3.6	20.5 ± 2.9	23.4 ± 3.3	23.4 ± 4.3
	21-30	20.6 ± 2.8	21.2 ± 3.8	23.6 ± 3.6* (↑14)	22.7 ± 3.7
	31-40	20.6 ± 3.4	21.0 ± 2.8	20.9 ± 2.4	21.8 ± 1.8
	41-50	20.6 ± 2.7	20.9 ± 2.4	20.8 ± 1.8	21.2 ± 2.7
PND 61	1-10	26.9 ± 5.1	32.5 ± 10.2	28.3 ± 6.6	26.7 ± 5.7
	11-20	23.1 ± 3.8	21.9 ± 3.5	23.1 ± 3.5	24.7 ± 2.8
	21-30	22.5 ± 2.1	22.8 ± 2.5	22.7 ± 2.8	24.3 ± 3.2
	31-40	23.6 ± 3.3	22.7 ± 1.8	24.1 ± 3.9	24.9 ± 4.5
	41-50	23.2 ± 4.7	22.7 ± 2.7	24.6 ± 3.5	24.1 ± 4.1
		Females			
PND 23	1-10	26.3 ± 4.6	28.4 ± 6.2	28.0 ± 5.3	25.9 ± 4.8
	11-20	22.5 ± 5.9	21.7 ± 4.7	24.8 ± 6.8	21.1 ± 2.1
	21-30	20.9 ± 4.3	21.5 ± 5.5	23.6 ± 6.4	20.7 ± 1.4
	31-40	20.9 ± 3.1	23.1 ± 6.0	21.8 ± 2.3	21.8 ± 2.9
	41-50	21.6 ± 3.4	23.7 ± 6.9	21.7 ± 3.5	21.0 ± 2.9
PND 61	1-10	25.9 ± 4.6	24.4 ± 3.6	26.9 ± 5.9	25.6 ± 6.2
	11-20	21.0 ± 2.4	21.1 ± 2.5	25.5 ± 6.6* (↑21)	21.8 ± 2.3
	21-30	20.6 ± 2.6	21.0 ± 4.3	25.0 ± 7.0* (↑21)	23.1 ± 2.8
	31-40	21.8 ± 2.9	23.3 ± 5.3	24.9 ± 5.0	22.9 ± 3.2
	41-50	21.9 ± 2.4	25.1 ± 4.7* (↑15)	22.6 ± 3.2	22.6 ± 3.4

a Data were obtained from Table 24 on pages 134-137 in MRID 46012925; n = 10-14. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

d) Learning and memory testing: No treatment-related differences were observed in the water maze tests at either age (Tables 10a and 10b). Swimming time in the Y-maze was sporadically increased or decreased during several trials in the 20 and 100 ppm males and in all dose groups in the females. However, these differences were transient and unrelated to dose. Swimming time in the straight channel was increased (↑23%; $p \leq 0.05$) in the 500 ppm females compared to controls during the memory phase (PND 62). There were no treatment-related differences in the percent of successful swimming trials in either sex at either time point (Table 10c). Numbers of trials to criterion, errors per trial, and animals that failed to learn were not reported.

TABLE 10a. Mean (± SD) swimming times (s) in water maze around weaning [a]

Session/Parameter		Dose (ppm)			
		0	20	100	500
Males					
Learning phase (PND 21)	Straight channel	4.49 ± 2.06	5.01 ± 2.70	4.47 ± 1.74	5.42 ± 2.54
	Trial 1	18.05 ± 7.97	15.65 ± 7.90	15.88 ± 8.31	21.14 ± 7.34
	Trial 2	10.29 ± 5.40	8.60 ± 4.78	12.64 ± 8.29	11.40 ± 5.27
	Trial 3	14.10 ± 6.49	9.92 ± 6.20* (↓30)	11.10 ± 6.97	12.43 ± 6.63
	Trial 4	10.35 ± 7.33	10.13 ± 5.51	9.99 ± 4.79	11.20 ± 7.33
	Trial 5	8.91 ± 4.01	6.56 ± 3.35	9.18 ± 4.52	9.50 ± 5.32
	Trial 6	9.22 ± 7.42	6.63 ± 3.85	9.39 ± 5.97	10.48 ± 6.29
Memory phase (PND 24)	Straight channel	3.46 ± 1.06	3.81 ± 2.51	3.41 ± 1.50	4.44 ± 2.20
	Trial 1	8.46 ± 3.46	9.16 ± 5.83	8.72 ± 5.63	7.40 ± 4.96
	Trial 2	5.33 ± 2.09	6.27 ± 4.48	5.30 ± 2.67	5.69 ± 2.61
	Trial 3	6.41 ± 4.13	4.44 ± 2.63* (↓31)	4.40 ± 3.07* (↓31)	5.37 ± 3.18
	Trial 4	5.55 ± 4.67	4.62 ± 2.51	5.52 ± 3.10	5.17 ± 2.55
	Trial 5	4.42 ± 2.07	4.81 ± 2.55	4.65 ± 2.15	3.89 ± 1.01
	Trial 6	5.33 ± 4.04	4.41 ± 1.63	5.08 ± 3.65	5.58 ± 2.88
Females					
Learning phase (PND 21)	Straight channel	4.05 ± 1.37	4.03 ± 1.50	5.17 ± 3.59	4.19 ± 1.70
	Trial 1	22.37 ± 6.76	14.27 ± 6.83** (↓36)	15.80 ± 6.39** (↓29)	17.48 ± 6.94* (↓22)
	Trial 2	10.57 ± 7.23	10.41 ± 7.23	12.42 ± 7.79	12.90 ± 6.90
	Trial 3	7.32 ± 4.36	8.91 ± 5.96	11.35 ± 7.23* (↑55)	11.17 ± 7.49* (↑53)
	Trial 4	6.80 ± 3.74	10.50 ± 5.93* (↑54)	8.83 ± 5.93	7.50 ± 5.69
	Trial 5	7.85 ± 4.99	8.12 ± 7.29	9.10 ± 6.11	7.00 ± 5.90
	Trial 6	10.04 ± 7.57	5.61 ± 3.15** (↓44)	7.92 ± 4.97	7.12 ± 5.96
Memory phase (PND 24)	Straight channel	4.08 ± 1.77	3.38 ± 1.12	3.74 ± 1.58	3.63 ± 1.24

Session/Parameter		Dose (ppm)			
		0	20	100	500
	Trial 1	7.83 ± 3.67	8.18 ± 5.85	8.63 ± 4.61	7.08 ± 3.29
	Trial 2	5.26 ± 3.49	3.87 ± 1.91	5.00 ± 2.67	4.94 ± 2.29
	Trial 3	4.01 ± 1.61	5.21 ± 3.23	5.36 ± 2.57	4.62 ± 3.45
	Trial 4	4.73 ± 2.43	5.37 ± 5.04	5.00 ± 3.71	3.76 ± 0.90
	Trial 5	5.49 ± 3.93	5.92 ± 4.66	5.36 ± 3.50	4.45 ± 2.48
	Trial 6	4.88 ± 2.92	7.08 ± 6.38	5.70 ± 2.96	5.09 ± 2.91

a Data were obtained from Table 25 on page 138-141 in MRID 46012925; n = 21-26. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

** Statistically different from controls at p≤0.01

*** Statistically different from controls at p≤0.001

TABLE 10b. Mean (± SD) swimming times (s) in water maze around PND 60 [a]

Session/Parameter		Dose (ppm)			
		0	20	100	500
Males					
Learning phase (PND 59)	Straight channel	4.05 ± 1.40	4.11 ± 1.50	5.04 ± 3.82	3.82 ± 0.88
	Trial 1	13.74 ± 5.69	12.96 ± 3.71	12.31 ± 4.63	11.96 ± 3.89
	Trial 2	6.26 ± 4.72	6.67 ± 4.04	6.84 ± 4.08	5.28 ± 1.96
	Trial 3	4.71 ± 2.34	4.84 ± 2.26	5.14 ± 3.08	4.97 ± 2.04
	Trial 4	4.91 ± 2.35	4.88 ± 2.09	4.79 ± 2.70	4.88 ± 2.00
	Trial 5	4.19 ± 2.19	5.79 ± 3.44* (↑38)	4.43 ± 1.45	5.44 ± 3.05
	Trial 6	4.63 ± 2.35	6.15 ± 4.00	4.81 ± 2.16	5.63 ± 3.65
Memory phase (PND 62)	Straight channel	3.14 ± 1.24	3.15 ± 1.16	3.02 ± 0.99	3.48 ± 1.17
	Trial 1	4.95 ± 2.26	5.41 ± 2.58	6.12 ± 3.44	5.08 ± 2.24
	Trial 2	4.12 ± 2.33	5.66 ± 5.98	3.76 ± 1.36	5.81 ± 5.18
	Trial 3	5.14 ± 3.43	6.81 ± 4.66	6.91 ± 4.02	6.42 ± 7.22
	Trial 4	5.52 ± 3.66	7.11 ± 5.63	6.67 ± 3.39	7.48 ± 5.41
	Trial 5	5.04 ± 2.32	7.60 ± 6.08	6.17 ± 4.01	7.47 ± 5.39
	Trial 6	6.11 ± 3.06	6.85 ± 5.42	6.33 ± 5.90	6.72 ± 4.54
Females					
Learning phase (PND 59)	Straight channel	4.33 ± 2.42	4.30 ± 2.33	4.34 ± 1.79	4.34 ± 2.75

Session/Parameter		Dose (ppm)			
		0	20	100	500
	Trial 1	12.93 ± 5.07	13.28 ± 5.00	14.45 ± 5.14	13.33 ± 5.58
	Trial 2	5.84 ± 3.27	5.18 ± 2.24	6.23 ± 4.15	7.05 ± 3.31
	Trial 3	5.08 ± 3.60	4.67 ± 1.76	5.31 ± 2.60	4.51 ± 2.05
	Trial 4	4.78 ± 2.69	5.26 ± 3.67	3.92 ± 1.61	5.79 ± 4.30
	Trial 5	4.15 ± 1.49	4.65 ± 2.71	4.01 ± 1.55	4.17 ± 1.26
	Trial 6	4.02 ± 2.26	4.32 ± 2.36	4.24 ± 2.25	3.86 ± 1.52
Memory phase (PND 62)	Straight channel	2.88 ± 0.73	3.14 ± 1.02	2.87 ± 0.69	3.55 ± 1.33* (↑23)
	Trial 1	4.35 ± 1.82	4.90 ± 2.29	4.44 ± 1.60	5.46 ± 2.80
	Trial 2	4.16 ± 2.73	6.94 ± 6.05* (↑67)	5.00 ± 3.52	5.45 ± 3.91
	Trial 3	6.35 ± 4.29	8.82 ± 7.35	4.23 ± 1.79	6.21 ± 6.36
	Trial 4	6.16 ± 4.91	8.15 ± 7.15	7.71 ± 4.93	7.95 ± 6.85
	Trial 5	5.59 ± 3.58	8.21 ± 5.49* (↑47)	6.18 ± 3.40	6.04 ± 3.74
	Trial 6	7.57 ± 7.07	9.70 ± 5.29	7.22 ± 5.82	6.73 ± 4.79

a Data were obtained from Table 25 on page 142-145 in MRID 46012925; n = 21-26. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

TABLE 10c. Mean (± SD) percent of successful swimming trials in water maze [a]

Session	Criterion	Dose (ppm)			
		0	20	100	500
		Males			
Learning phase (PND 21)	≤ 10 seconds	50.0 ± 22.4	64.7 ± 23.7* (↑29)	51.9 ± 21.8	45.8 ± 18.6
	≤ 2 x straight channel time	37.3 ± 29.8	54.7 ± 28.7* (↑47)	43.2 ± 26.7	45.8 ± 27.0
Memory phase (PND 24)	≤ 10 seconds	88.1 ± 13.1	90.7 ± 13.7	88.3 ± 10.1	91.0 ± 11.0
	≤ 2 x straight channel time	70.6 ± 20.3	70.7 ± 22.7	68.5 ± 22.8	80.6 ± 18.2
Learning phase (PND 59)	≤ 10 seconds	81.7 ± 12.8	75.7 ± 16.3	82.6 ± 14.3	82.5 ± 12.3
	≤ 2 x straight channel time	72.2 ± 19.2	70.8 ± 16.5	78.5 ± 15.1	70.6 ± 19.7
Memory phase (PND 62)	≤ 10 seconds	92.1 ± 10.0	84.7 ± 19.6	86.8 ± 14.7	83.3 ± 21.7
	≤ 2 x straight channel time	69.0 ± 26.0	63.9 ± 22.3	63.9 ± 21.8	66.7 ± 29.3
		Females			
Learning phase (PND 21)	≤ 10 seconds	59.5 ± 22.1	63.3 ± 18.0	54.3 ± 16.4	57.6 ± 17.0
	≤ 2 x straight channel time	50.8 ± 27.1	58.0 ± 25.1	52.5 ± 26.4	51.4 ± 20.2
Memory phase (PND 24)	≤ 10 seconds	88.1 ± 14.1	85.3 ± 18.2	87.0 ± 13.3	91.7 ± 8.5
	≤ 2 x straight channel time	79.4 ± 21.0	70.7 ± 26.5	71.0 ± 21.0	79.2 ± 15.7
Learning phase (PND 59)	≤ 10 seconds	82.5 ± 13.4	81.7 ± 13.8	83.3 ± 11.5	83.3 ± 10.1
	≤ 2 x straight channel time	74.6 ± 18.7	76.2 ± 13.5	75.0 ± 19.0	73.2 ± 21.2

Session	Criterion	Dose (ppm)			
		0	20	100	500
Memory phase (PND 62)	≤ 10 seconds	85.7 ± 18.5	75.4 ± 22.7	88.5 ± 13.1	87.0 ± 22.4
	≤ 2 x straight channel time	73.8 ± 22.7	50.8 ± 28.6** (↓31)	62.2 ± 22.9	67.4 ± 22.2

a Data were obtained from Table 26 on page 146-161 in MRID 46012925; n = 21-27. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Statistically different from controls at p≤0.05

** Statistically different from controls at p≤0.01

5. Postmortem results

a) Organ weights: At 500 ppm, absolute and adjusted for body weight liver weights were increased (↑20-51%; p≤0.01) in males and females at PND 5 and 12 (Table 11). Relative (to body) liver weights were also increased (↑26-36%; statistics not performed) in these animals at PND 5 and 12.

There were no effects of treatment on brain weights (Table 12). Absolute brain weights were decreased in the 100 ppm males and 500 ppm females on PND 63 (↓3%; p≤0.05). However, these differences from controls were considered unrelated to treatment because they were minor and were not dose-related.

TABLE 11. Mean (±SD) liver weights in F_1 rats [a]

Post-natal Day	Parameter		Dose (ppm)	
			0	500
Males				
PND 5	Terminal body weight (g)		9.6 ± 1.5	9.4 ± 1.5
	Liver	absolute (g)	0.318 ± 0.063	0.403 ± 0.072** (↑27)
		relative to bw (%)	3.324 ± 0.330	4.292 ± 0.378
		adjusted for bw	0.314	0.406** (↑29)
PND 12	Terminal body weight (g)		24.9 ± 1.6	20.6 ± 2.9
	Liver	absolute (g)	0.816 ± 0.083	0.978 ± 0.154** (↑20)
		relative to bw (%)	3.276 ± 0.238	4.748 ± 0.310
		adjusted for bw	0.717	1.077** (↑50)
Females				
PND 5	Terminal body weight (g)		9.1 ± 1.3	8.9 ± 1.6
	Liver	absolute (g)	0.314 ± 0.052	0.386 ± 0.074** (↑23)
		relative to bw (%)	3.460 ± 0.219	4.356 ± 0.466
		adjusted for bw	0.310	0.390** (↑26)
PND 12	Terminal body weight (g)		23.2 ± 3.3	20.8 ± 2.0

Post-natal Day	Parameter	Dose (ppm)	
		0	500
	Liver absolute (g)	0.753 ± 0.110	1.022 ± 0.139** (↑36)
	relative to bw (%)	3.247 ± 0.226	4.897 ± 0.397
	adjusted for bw	0.701	1.061** (↑51)

a Data obtained from Table 29 on pages 164-165 in MRID 46012925. Percent difference from controls, calculated by the reviewers, is included in parentheses.
n = 21-27 on PND5, and n = 10-13 on PND 12

** Statistically different from controls at p≤0.01

** Statistically different from controls at p≤0.01

TABLE 12. Mean (±SD) brain weights in F_1 rats [a]

Post-natal Day	Parameter	Dose (ppm)			
		0	20	100	500
	Males				
PND 12	Terminal body weight (g)	24.9 ± 1.6	23.7 ± 2.6	23.7 ± 1.9	20.6 ± 2.9
	Brain absolute (g)	1.13 ± 0.05	1.15 ± 0.05	1.14 ± 0.05	1.10 ± 0.09
PND 63	Terminal body weight (g)	364.3 ± 20.6	351.4 ± 24.1	354.7 ± 25.2	343.3 ± 18.8
	Brain absolute (g)	2.08 ± 0.05	2.03 ± 0.07	2.02 ± 0.06* (↓3)	2.03 ± 0.06
	Females				
PND 12	Terminal body weight (g)	23.2 ± 3.3	22.5 ± 2.8	23.4 ± 1.9	20.8 ± 2.0
	Brain absolute (g)	1.10 ± 0.06	1.10 ± 0.05	1.10 ± 0.04	1.08 ± 0.04
PND 63	Terminal body weight (g)	223.0 ± 17.5	237.3 ± 9.2	220.5 ± 18.5	211.5 ± 13.6
	Brain absolute (g)	1.91 ± 0.04	1.94 ± 0.05	1.91 ± 0.05	1.86 ± 0.05* (↓3)

a Data obtained from Table 30 on pages 166-168 in MRID 46012925. Percent difference from controls, calculated by the reviewers, is included in parentheses.
n = 10-14

* Statistically different from controls at p≤0.05

b) Neuropathology

1) Macroscopic examination: No treatment-related findings were noted. Examination of the uterus revealed that the two females that did not litter (one control and one 50 ppm) were not pregnant.

2) Microscopic examination: The microscopic examinations of brains morphometry on mid- and low-dose groups are presented below in Table 13.

Table 13: Mean Brain Morphometric Measurement in PND 12 and PND 63 Offspring

Measurement	Sex	Dietary concentration Clodinafop-propargyl (ppm)								Historical Control
		0	20	100	500	0	20	100	500	
		Postnatal Day 12				Postnatal Day 63				
3DB Level 3 – Hippocampus – Length from Midline	M	2.79	2.61** (6)	2.68	2.67	2.62	2.53	2.57	2.69	2.58-3.31
	F	2.83	2.58** (9)	2.67* (6)	2.61** (8)	2.62	2.60	2.57	2.58	
4C Level 4 – Corpus Callosum – Thickness	M	0.58	0.58	0.60	0.56	0.38	0.38	0.36	0.37	0.49-0.654 (PND 12)
	F	0.64	0.56** (13)	0.58** (9)	0.59* (8)	0.34	0.38	0.36	**0.42**** **(24)**	0.304-0.453 (PND 63)
4HB Level 4 – Hippocampus – Width Dentate Gyrus	M	0.48	0.44** (8)	0.45** (6)	0.46	0.63	0.59* (6)	0.58** (8)	0.62	0.451-0.577
	F	0.49	0.44** (10)	0.45** (8)	0.46** (6)	0.62	0.58** (6)	0.58** (6)	0.63	
5AB Level 5 – Dorsal Cortex – Thickness	M	1.06	1.08	1.06	1.08	1.38	1.35	1.31* (5)	1.32* (4)	1.19-1.41
	F	1.12	1.05** (6)	1.06** (5)	1.10	1.30	1.30	1.28	1.34	
5BB Level 5 – Piriform Cortex – Thickness	M	1.08	1.07	1.08	1.11	1.22	1.19	1.21	1.11** (9)	1.06-1.26
	F	1.10	1.07	1.06	1.09	1.21	1.19	1.20	1.22	
8H Level 8 – Cerebellum Height	M	3.53	3.52	3.49	3.70** (5)	5.74	5.30** (8)	5.36* (7)	- 5.75	3.42-3.91
	F	3.66	3.55	3.58	3.74	5.43	5.18	5.28	5.60	

** Statistically significantly different from control ($p<0.01$)
* Statistically significantly different from control ($p<0.05$)
- Not statistically significant ($p>0.05$)
() Values in parentheses are the percentage difference from control

- Hippocampus length: On PND 12, the level 3 length from midline in the hippocampus was decreased (↓6-9%) at all dose levels in females, but there was no dose response. Mean values in males at 100 and 500 ppm were comparable to control values. At 20 ppm, the mean value was decreased (6%). On PND 63, no significant differences were seen in any treatment group, in either male or female. The mean value for males at 20 ppm was somewhat lower than the historical control range. Mean values for males and females at 100 ppm were only slightly below the historical control range.
- Corpus callosum thickness: On PND 12, level 4 thickness of the corpus callosum was decreased (8-13%) at all dose levels in females in the absence of a dose response Mean values in males at all treated groups were comparable to concurrent control values. Mean values for males and females were all within the

historical control range. On PND 63, mean corpus callosum thickness was significantly ($p < 0.001$) increased (24%) at the high dose females when compared to controls. No significant differences were seen in females at the low- and mid dose nor in males at any dose level.

- Hippocampus width at the dentate gyrus: On PND 12, the level 4 width of the dentate gyrus in the hippocampus was decreased (6-10%) at all dose levels in females in the absence of a dose response. In males, the decreases were seen only at the low and mid dose groups. On PND 63, differences showed statistical significance at the low and mid dose groups but not at the high dose in both sexes. Additionally, the mean values were all above the historical control range.
- Dorsal cortex thickness: On PND 12, level 5 thickness of the dorsal cortex was decreased in females at the low (6%) and mid (5%) dose groups but not at the high dose. Mean values in males at all treated groups were comparable to control values. On PND 63, decreases were seen in males at the mid (5%) and high (4%) dose groups. Mean values in females at all treated groups were comparable to concurrent control values. For PN63, the mean values were within the historical control range. At PN12, mean values for both male and females, at all doses and in controls, were below the historical control range.
- Piriform cortex thickness: On PND 12, no significant differences were seen in any treatment group, in either male or female. On PND 63, except for a 9% decrease at the high dose males, the mean value of which was within the historical control range, no significant differences were seen in males at the low and mid dose nor in females at any dose level. Mean values for both sexes at all doses were within the historical control range at both PND12 and PND63.
- Cerebellum height: On PND 12, there was a statistically significant ($p<0.001$) increase (5%) in the cerebellum height at the high dose males; no differences were seen in males at the lower dose nor in females at any dose level. On PND 63, decreases were seen in males at the low (8%) and mid (7%) dose groups but not at the high dose. No differences were seen in females at any dose level.

Most of the changes observed in the various regions of the brain were not considered to be treatment related given the lack of dose response, the relatively small magnitude of change, lack of consistency between the time periods and the fact that the mean values were within the range of historical control means. In contrast, due the magnitude (24%) and pair-wise significance ($p < 0.001$) the increase observed in the level 4 thickness of the corpus callosum at the high dose in PND 63 females is considered to be treatment related.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the maternal LOAEL was 500 ppm based on decreased body weights and food consumption during lactation. The LOAEL for the offspring was 500 ppm based on body weights decreased (↓18-19%) compared to controls. In addition, liver weights were markedly higher than controls at 500 ppm. Some differences in development were observed in the offspring that were attributed to the decreased

body weights and not a direct developmental effect. No evidence of developmental neurotoxicity was observed at doses up to 500 ppm.

B. REVIEWER COMMENTS

In a developmental neurotoxicity study, Clodinafop-propargyl was administered in the diet to pregnant Alpk:APfSD Wistar-derived rats (30/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 20, 100, or 500 ppm (equivalent to 0/0, 1.8/3.5, 9.0/18.0, and 44.0/85.5 mg/kg/day [gestation/lactation]). Dams were allowed to deliver naturally and were killed on LD 29. On postnatal day (PND) 5, litters were standardized to 8 pups/litter; the remaining offspring and dams were sacrificed and discarded without further examination. Subsequently, 1 pup/litter/group (at least 10 pups/sex/dose when available) were allocated to subsets for FOB, motor activity, acoustic startle response, learning and memory evaluation, and neuropathological examination.

For maternal toxicity, no treatment-related effects were observed in mortality, clinical signs, body weight, body weight gains, food consumption, reproductive performances or post mortem examinations.

The maternal NOAEL is 500 ppm (44 mg/kg/day). The maternal LOAEL was not established.

For offspring, no significant treatment-related differences in live litter size, post-natal survival or sex ratios were observed. Pre-weaning pup body weights were decreased (↓9-19%; $p \leq 0.01$) in both sexes at 500 ppm beginning on PND 12 through PND 22. Minor decreases (↓2-5%; $p \leq 0.05$) in body weights were noted in the 100 ppm males on PND 22 and in the 20 ppm and 100 ppm females on PND 18 and 22. Post-weaning body weights remained decreased (↓4-15%; $p \leq 0.01$) in the males through termination on PND 63 and in the females through PND 57. Minor decreases (↓2-4%; $p \leq 0.05$) in body weights were noted in the 100 ppm males through PND 50 and in the 100 ppm females through PND 36.

For sexual maturation, increased ($p \leq 0.05$) time to preputial separation was noted in the 100 and 500 ppm male pups when compared to controls (45.5-45.7 days treated vs 44.7 days controls). Time to vaginal opening was delayed in the 500 ppm females (35.6 days) compared to controls (34.6 days). However, it was not considered biologically significant since these results were within the historical control ranges.

Motor activity assessment was determined to be inadequate because of the lack of habituation in the female control groups confounded the interpretation of the effects seen in the low and mid dose group female offspring.

Treatment-related effects on auditory startle reflex were observed at high dose on PND 23 in peak amplitude with supportive responses observed at the mid-dose. There were no treatment-related differences in the water maze tests. Swimming time in the Y-maze was sporadically increased or decreased during several trials in the 20 and 100 ppm males and in all dose groups

in the females. However, these differences were transient and unrelated to dose. Swimming time in the straight channel was increased (↑23%; p≤0.05) in the 500 ppm females compared to controls during the memory phase (PND 62). There were no treatment-related differences in the percent of successful swimming trials in either sex at either time point

For postmortem examinations, absolute liver weights were increased (↑20-36%; p≤0.01) at 500 ppm in males and females at PND 5 and 12. Relative (to body) liver weights were also increased (↑26-51%; statistics not performed) in these animals at PND 5 and 12.

Evaluation of the brain morphometric data at all dose levels showed several measurements that were significantly different (p≤0.05) from the concurrent controls. However, most of the changes were determined not to be treatment related due to lack of dose-, time, and/or sex-response and the mean values were within the range of the historical control means. In contrast, the 24% increase (p <0.01) seen in the level 4 corpus callosum thickness in PND 63 females at the high dose was considered to be treatment-related due to the magnitude of the increase and changes in linear morphometric measurements can reflect alterations in the development of particular brain regions that are associated with functional deficits. The corpus callosum captures several major developmental processes subject to environmental insult, including myelination, axonal growth and pruning. For example, a loss of the pruning process may result in a larger corpus callosum than is typical (Altman 1987; Rodier 1988, 1995, 2004; Rodier et al 1997).

The offspring LOAEL is 500 ppm (44.0 mg/kg/day) based on decreased pup body weights and body weight gains, increased liver weight, decreased auditory response on PND 23 and increased thickness of corpus callosum in females on PND 63. The offspring NOAEL is 100 ppm (9.0 mg/kg/day)

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 due to the inadequacies in the assessment of motor activity in the offspring and the pending review of the positive control data.

C. **STUDY DEFICIENCIES:** The following study deficiencies were noted; however, these deficiencies would not affect the conclusion of this study.

- Physical landmarks (incisor eruption, eye opening) were not evaluated.
- F1 survival data past PND 5 were not provided.
- Detailed descriptions of the apparatuses for measuring motor activity and auditory startle were not provided.

Appendix I

Range-finding study for developmental neurotoxicity of Clodinafop-propargyl in rats

In a range-finding study (MRID 46012948), Clodinafop-propargyl (94.2% a.i., Batch #P903007) was administered in the diet to pregnant Alpk:AP$_f$SD Wistar-derived rats (10/dose) from gestation day (GD) 7 to lactation day (LD) 22 at nominal doses of 0, 50, 1000, or 1500 ppm (equivalent to 0/0, 41.9/82.5, 78.1/146.3, and 118.4/201.8 mg/kg/day [gestation/lactation]). Dams were allowed to deliver naturally and were sacrificed on LD 22. Clinical signs, body weight, and food consumption were recorded for the dams. The number, survival, clinical condition, and body weights of the pups were monitored. No pups were culled to standardize the litter size. Liver weights were recorded in the pups on PND 5, 12, and 22 and in the dams on LD 22. Functional observational battery (FOB), motor activity, acoustic startle response, learning and memory, and neuropathology were not examined in the pups.

There were no treatment-related clinical observations in the dams.

Maternal body weights (adjusted for initial body weight) were decreased ($p \leq 0.05$) at 1000 ppm beginning on lactation day (LD) 12 (↓4-6%) and at 1500 ppm beginning on LD 5 (↓5-12%). Maternal food consumption was decreased ($p \leq 0.05$) at 500 ppm on LD 15-22 (↓11%), at 1000 ppm on LD 8-22 (↓17-25%), and at 1500 ppm on LD 8-22 (↓28-38%). Absolute and adjusted (for body weight) liver weights were increased ($p \leq 0.01$) at 500 (↑14-15%), 1000 (↑22-27%), and 1500 (↑18-31%) ppm. The percentage of pups born live was decreased ($p \leq 0.05$) at 1500 ppm (95.7%) compared to controls (100.0%). Whole litter losses were increased ($p \leq 0.05$) at 1500 ppm (2/10 dams) compared to controls (0/10 dams).

Pup survival was decreased ($p \leq 0.01$) at 1500 ppm (68.7%) compared to controls (98.3%) on PND 5. Litter size, excluding whole litter losses, was decreased (↓26-39%; $p \leq 0.01$) at this dose throughout the post-natal period beginning on post-natal day (PND) 5. Adjusted body weights of the pups were decreased ($p \leq 0.01$) in both sexes at 500 ppm on PND 22 (↓14-16%), at 1000 ppm on PND 15 and 22 (↓11-25%), and at 1500 ppm on PND 12, 15, and 22 (↓9-26%). Total litter weight was decreased ($p \leq 0.05$) at 500 ppm on PND 22 (↓23%) and at 1000 and 1500 ppm throughout the post-natal period (↓13-57%). Aside from the numbers of pups found dead or missing and presumed dead at 1500 ppm, more pups at this dose were cold (10 pups in 4 litters) compared to controls (3 pups in 2 litters) and were small (5 pups in 2 litters) compared to controls (1 pup). Pup liver weights were increased ($p \leq 0.05$) over controls as follows: (i) adjusted weight at ≥500 ppm and absolute weight at ≥1000 ppm in females on PND 5 (↑29-58%); (ii) absolute and adjusted weights at ≥500 ppm in both sexes on PND 12 (↑40-77%); and (iii) adjusted weight in both sexes on PND 22 (↑51-57%).

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

REFERENCES

Altman J. (1987). Morphological and Behavioral Markers of Environmentally Induced Retardation of Brain Development: An Animal Model. Environ Health Perspectives 74:153-168.

Rodier P.M. (1988). Structural--functional relationships in experimentally induced brain damage. Prog Brain Res 73:335-348.

Rodier P.M. (1995). Developing Brain as a Target of Toxicity. Environ Health Perspect 103(Suppl 6):73-76.

Rodier P.M., Ingram J.L., Tisdale B., and Croog V.J. (1997). Linking etiologies in humans and animal models: studies of autism. Reprod Toxicol 11:417-422.

Rodier P.M. (2004). Environmental Causes of Central Nervous System Maldevelopment. Pediatrics 113:1076-1083.

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